

# A variant of mitochondrial protein LOC387715/ARMS2, not HTRA1, is strongly associated with age-related macular degeneration

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Genetic variants at chromosomes 1q31-32 and 10q26 are strongly associated with susceptibility to age-related macular degeneration (AMD), a common blinding disease of the elderly. We demonstrate, by evaluating 45 tag SNPs spanning *HTRA1*, *PLEKHA1*, and predicted gene *LOC387715/ARMS2*, that rs10490924 SNP alone, or a variant in strong linkage disequilibrium, can explain the bulk of association between the 10q26 chromosomal region and AMD. A previously suggested causal SNP, rs11200638, and other examined SNPs in the region are only indirectly associated with the disease. Contrary to previous reports, we show that rs11200638 SNP has no significant impact on *HTRA1* promoter activity in three different cell lines, and *HTRA1* mRNA expression exhibits no significant change between control and AMD retinas. However, SNP rs10490924 shows the strongest association with AMD ( $P = 5.3 \times 10^{-30}$ ), revealing an estimated relative risk of 2.66 for GT heterozygotes and 7.05 for TT homozygotes. The rs10490924 SNP results in nonsynonymous A69S alteration in the predicted protein LOC387715/ARMS2, which has a highly conserved ortholog in chimpanzee, but not in other vertebrate sequences. We demonstrate that *LOC387715/ARMS2* mRNA is detected in the human retina and various cell lines and encodes a 12-kDa protein, which localizes to the mitochondrial outer membrane when expressed in mammalian cells. We propose that rs10490924 represents a major susceptibility variant for AMD at 10q26. A likely biological mechanism is that the A69S change in the LOC387715/ARMS2 protein affects its presumptive function in mitochondria.

aging | genetic association | mitochondria | neurodegeneration | retinal disease

Age-related macular degeneration (AMD) is a common disorder that primarily affects the central region of the retina (macula) and is a leading cause of blindness in the elderly. Early symptoms of the disease are characterized by the presence of ophthalmoscopically visible soft drusen, with areas of hyper- or depigmentation, whereas later stages manifest as either choroidal neovascularization or atrophy of photoreceptors and retinal pigment epithelium (RPE) (1–3). Susceptibility to AMD is a multifactorial trait involving both genetic (4–6) and environmental factors (7, 8); however, its precise etiology remains elusive. A number of AMD-associated sequence variants exhibiting small effects have been reported in genes including *ATP-binding transporter protein 4 (ABCA4)* (9), *apolipoprotein E (APOE)* (10), *excision-repair cross-complementing group 6 (ERCC6)* (11), *fibulin 5 (FBLN5)* (12), *elongation of very-long-chain fatty acids-like 4 (ELOVL4)* (13), *factor B/complement component 2 (BF/C2)* (14), *toll-like receptor 4 (TLR4)* (15), and *vascular endothelial growth factor (VEGF)* (16).

Genome-wide linkage studies have revealed disease susceptibility haplotypes of large effect at chromosomes 1q31-32 and 10q26 (6). In a remarkable example of the convergence of alternative approaches for gene mapping, independent research efforts identified the Y402H variant in *complement factor H (CFH)* on chro-

somosome 1q32 as the first major AMD-susceptibility allele (7, 17–20). A second genomic region with similarly consistent linkage evidence is reported at chromosome 10q26 (6), where rs10490924 and nearby SNPs that span a 200-kb region of linkage disequilibrium show strong association to AMD (8, 21, 22). A consensus from multiple studies is that the 10q26 region harbors a second major genetic determinant of AMD susceptibility (8, 21–23). Markers showing evidence of association at 10q26 overlap with three genes, *PLEKHA1*, *LOC387715/ARMS2* (*age-related maculopathy susceptibility 2*), and *HTRA1/PRSS11* (*high-temperature requirement factor A1*). *PLEKHA1* has a pleckstrin homology domain, whereas *LOC387715/ARMS2* encodes a hypothetical protein of unknown function. It was initially proposed that polymorphisms in the region alter the risk of AMD by modulating the function of one of these two genes (8, 21, 22). More recently, two reports proposed a causal relationship between AMD susceptibility and rs11200638, another SNP in the same 200-kb region of 10q26, and suggested that this promoter variant affects the expression of a serine protease *HTRA1/PRSS11* (24, 25). This interpretation contrasts with other reports (8, 21–23), which find the strongest association with rs10490924; T allele of rs10490924 maps to exon 1 of the hypothetical *LOC387715/ARMS2* gene and changes putative amino acid 69 from alanine to serine.

To resolve the apparently contradictory reports, we undertook a detailed association analysis of SNPs at 10q26. Here, we show that the observed strong association of AMD susceptibility to rs10490924 cannot be explained by rs11200638 and that the region surrounding the rs11200638 variant does not bind to AP-2 $\alpha$  transcription factor and has no significant effect on *HTRA1* mRNA expression. Instead, the rs10490924 variant alters the coding sequence of an apparently primate-specific gene *LOC387715/ARMS2*, which we show can produce a protein that localizes to the mitochondria when expressed in mammalian cells. Taken together, our results suggest that changes in the activity or regulation of *LOC387715/ARMS2* are likely responsible for the impact of rs10490924 on AMD disease susceptibility and that the association with rs11200638 is likely to be indirect.

## Results

**Association Analysis.** To examine the association of rs10490924, rs11200638, and neighboring variants with AMD, we first geno-

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The authors declare no conflict of interest.

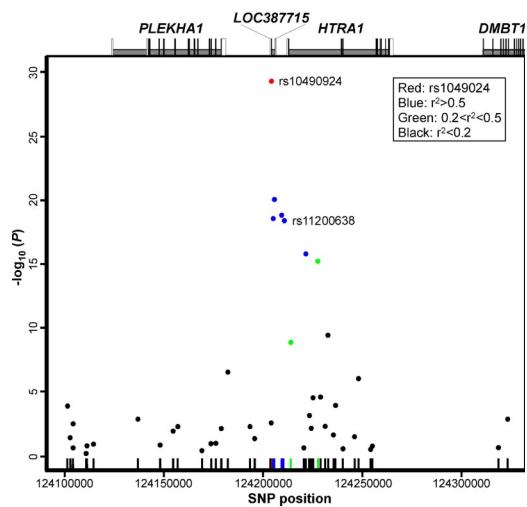
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Abbreviations: AMD, age-related macular degeneration; RPE, retinal pigment epithelium.

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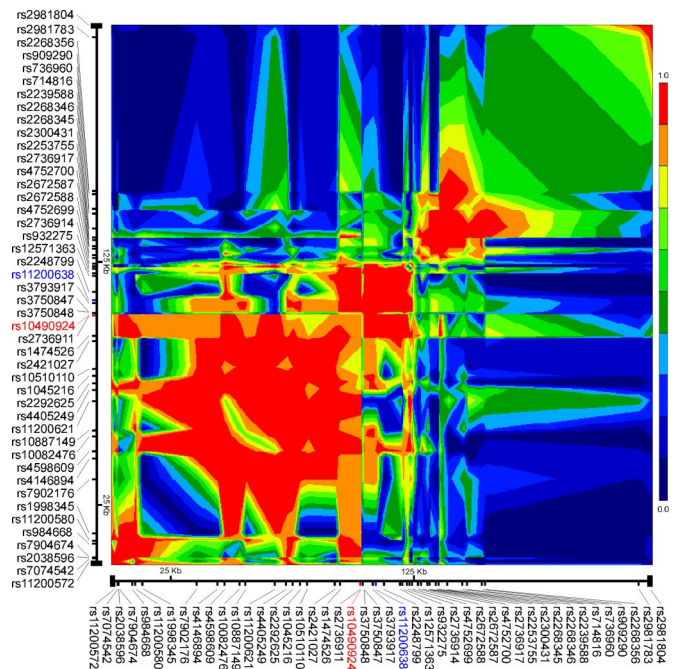
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**Fig. 1.** Association analysis of the 10q26 chromosomal region. Shown are  $P$  values for single SNP association tests comparing unrelated cases and controls. The genes in the indicated region are *PLEKHA1*, *LOC387715/ARMS2*, *HTRA1*, and *DMBT1*. rs1049024, the SNP showing strongest association in the region, is colored in red. Markers in strong association are colored in blue ( $r^2 > 0.5$ ) or green ( $r^2 > 0.3$ ).

typed these two and an additional 43 SNPs in 466 AMD cases and 280 controls from our cohort, which has been described elsewhere (20, 26). The SNPs were selected to capture 172 common polymorphisms characterized by the HapMap consortium (27) in the 220-kb region spanning *PLEKHA1*, *LOC387715/ARMS2*, and *HTRA1* with an average  $r^2$  of 0.92. Our results are summarized in Figs. 1 and 2, Table 1 (top 10 SNPs), and [supporting information \(SI\) Tables 2 and 3](#). After fitting a parametric association model (26, 28), marker rs1049024 showed the strongest association with AMD ( $P = 5.3 \times 10^{-30}$ ), with an estimated relative risk of 2.66 for GT heterozygotes and 7.05 for TT homozygotes. As expected, the risk allele T has a significantly higher frequency in cases than in controls (51.7% vs. 22.0%,  $P < 10^{-28}$ ). Four other SNPs (rs3750847, rs3793917, rs3750848, and rs11200638) show strong but less-significant association ( $10^{-21} < P < 10^{-18}$ ). In particular, the rs11200638 SNP showed a weaker association ( $P = 3.8 \times 10^{-19}$ ) with an estimated relative risk of 2.21 for AG heterozygotes and 4.87 for AA homozygotes. All of the five listed SNPs are in high-linkage disequilibrium (Fig. 1 and [SI Table 4](#)). Using logistic regression to evaluate models with two or more SNPs, we found that when rs1049024 was included no other SNP showed significant evidence for association (rs2253755 had the strongest association after accounting for rs1049024,  $P = 0.027$ , which is nonsignificant after adjusting for multiple testing). In contrast, when rs11200638 or any other SNP was used to seed the model, rs1049024 still showed significant evidence for association ( $P < 10^{-6}$  or less, depending on the SNP used to seed the model). Overall, our genetic data are consistent with a model in which rs1049024 alone, or another ungenotyped SNP in very strong disequilibrium with it, is directly responsible for association with AMD. In addition, our results suggest that rs11200638 and the other examined SNPs are only indirectly associated with the disease. Our data does not support a model in which rs11200638 alone explains the association of the 10q26 region with macular degeneration.

In addition to a multiplicative model with one degree of freedom (as outlined above), we also fitted two degree of freedom models to the data. These models did not significantly improve fit ( $P > 0.1$ ) and did not lead to qualitatively different conclusions. In particular, the data still led to the conclusion that rs1049024 was the strongest associated SNP in the region and that association with any other



**Fig. 2.** Graphical overview of linkage disequilibrium (LD) among 45 SNPs. The plot summarizes the linkage disequilibrium ( $D'$ ) between all pairs of SNPs in the region. Pairs of SNPs showing strong linkage disequilibrium ( $\approx 0.70$  or greater) are colored in red and orange. Intermediate levels of disequilibrium ( $\approx 0.30$ – $0.70$ ) are colored in green, and lower levels of disequilibrium are colored in blue.

SNP could be accounted for by rs1049024. These two degrees of freedom models also did not support the possibility that rs11200638 is the major determinant of disease susceptibility in the region.

**Effect of rs11200638 on *HTRA1* Expression.** To follow-up our genetic analysis, we examined the impact of the previously proposed causal variant rs11200638 on *HTRA1* expression and investigated the potential roles of *LOC387715/ARMS2*, the hypothetical gene whose coding sequence is altered by rs1049024. The SNP rs11200638 is located within a conserved genomic region upstream of human and mouse *HTRA1* genes (Fig. 3A). To evaluate previous reports (24, 25) of the effects of SNP rs11200638 on *HTRA1* promoter activity, we generated mammalian expression constructs carrying three different lengths of the WT *HTRA1* promoter (WT-long, -medium, and -short) and the mutant sequence carrying the AMD-risk allele at the SNP rs11200638 (SNP-long and -medium). These constructs were transfected into HEK293, ARPE-19 (human RPE), and Y79 (human retinoblastoma) cells; in all three cell lines, WT and variant SNP promoter activities did not show statistically significant differences in the luciferase reporter expression, and the WT-short promoter (not including rs11200638 region) showed higher transcriptional activities than the others (Fig. 3B–D).

Although the rs11200638 region includes several transcription factor binding sites as suggested by *in silico* analysis (Fig. 3E), Dewan *et al.* (24) focused on putative binding sites for transcription factors activating enhancer-binding protein-2 $\alpha$  (AP-2 $\alpha$ ) and serum-response factor. EMSA did not detect any supershift of the nucleotide sequence spanning rs11200638 variation with anti-AP-2 $\alpha$  antibody (Fig. 3F, lane 5). Among the transcription factors examined, only stimulating protein 1 (SP-1) antibody produced a weakly shifted DNA–protein complex (Fig. 3F, lane 6). We also note that quantitative RT-PCR analysis provided suggestive evidence for a decrease in *HTRA1* expression in AMD retinas (similar threshold levels after an average of  $21.6 \pm 0.6$  RT-PCR cycles in control retinas versus  $22.2 \pm 0.3$  cycles in AMD retinas; four independent retinas examined in quadruplicate for each). This finding contrasts



**Table 1. SNPs showing the strongest association with AMD**

SNP	Allele		Risk allele frequency	lod, 1 df	P	Penetrances			$\lambda_{sib}$	RR1	RR2	Logistic regression, including covariates for the following	
	Risk (-)	Nonrisk (+)				+/+	+/-	-/-				rs10490924	rs11200638
rs10490924	T	G	0.284	28.12	$5.3 \times 10^{-30}$	0.092	0.245	0.652	1.275	2.656	7.054	NA	$6.27 \times 10^{-8}$
rs3750847	T	C	0.247	18.95	$9.4 \times 10^{-21}$	0.114	0.264	0.61	1.191	2.313	5.347	0.067	0.003
rs3793917	G	C	0.243	17.75	$1.6 \times 10^{-19}$	0.116	0.265	0.603	1.182	2.275	5.174	0.051	0.002
rs3750848	G	T	0.246	17.45	$3.1 \times 10^{-19}$	0.117	0.263	0.594	1.178	2.253	5.080	0.103	0.008
rs11200638	A	G	0.248	17.36	$3.8 \times 10^{-19}$	0.118	0.262	0.577	1.167	2.207	4.872	0.241	NA
rs932275	A	G	0.235	14.75	$1.7 \times 10^{-16}$	0.12	0.269	0.601	1.172	2.238	5.007	0.140	0.050
rs2672587	G	C	0.254	14.19	$6.3 \times 10^{-16}$	0.123	0.256	0.534	1.142	2.086	4.351	0.137	0.124
rs2253755	G	A	0.312	8.54	$3.6 \times 10^{-10}$	0.132	0.23	0.398	1.078	1.736	3.014	0.027	0.017
rs2248799	T	C	0.494	7.97	$1.4 \times 10^{-9}$	0.112	0.188	0.316	1.066	1.680	2.820	0.307	0.421
rs10510110	C	T	0.51	5.75	$2.7 \times 10^{-7}$	0.116	0.187	0.302	1.055	1.612	2.599	0.347	0.531

For each SNP, the risk allele (-) is defined as the allele with increased frequency in affected individuals. Evidence for association, as evaluated by the LAMP program (26), is summarized through the risk allele frequency in the population (estimated using a parametric model that, in effect, weights cases and controls according to the estimated disease prevalence); lod (logarithm of odds) score ( $\log_{10}$  likelihood ratio statistic comparing model with and without association); P value; and a series of estimated penetrances for nonrisk homozygotes (+/+), heterozygotes (+/-), and risk allele homozygotes (-/-); genotype relative risks RR1 and RR2 (which are computed by comparing estimated penetrances in heterozygotes and risk-allele homozygotes, respectively, and those for nonrisk homozygotes); and sibling recurrence risks  $\lambda_{sib}$ . The  $\lambda_{sib}$  measure characterizes the overall contribution of a locus to disease susceptibility. It quantifies the increase in risk to siblings of affected individuals attributable to a specific locus (48). For example,  $\lambda_{sib}$  of 1.27 signifies that the SNP could account for 27% in risk of AMD for relatives of affected individuals. Association analysis using a simple  $\chi^2$  statistic produced similar results. The last two columns summarize P value results of logistic regression analysis, including either rs10490924 or rs11200638 as covariates. As suggested in ref. 40, missing genotypes were imputed prior to the sequential analyses reported in the last two columns.

with the smaller original experiment suggesting an increase in HTRA1 expression in lymphocytes from AMD patients ( $P = 0.02$ ) (24, 25). Taken together, the data indicate no significant change in HTRA1 expression between AMD patients and controls.

**Expression and Subcellular Localization of LOC387715/ARMS2.** We investigated the possible role of LOC387715/ARMS2, the hypothetical gene whose coding sequence is altered by rs10490924. LOC387715/ARMS2 encodes a predicted human protein with a highly conserved ortholog in chimpanzee, but not in other mammals or vertebrates (Fig. 4A). The T allele of SNP rs10490924 is predicted to result in a coding change (A69S) of the LOC387715/ARMS2 protein. This alanine-to-serine substitution creates a new putative phosphorylation site and breaks a predicted  $\alpha$ -helix (Fig. 4A).

RT-PCR analysis showed that LOC387715/ARMS2 mRNA is expressed abundantly in JEG-3 (human placenta choriocarcinoma) and faintly in the human retina and other cell lines, whereas HPRT (control) transcript is detected to a similar degree in all tissues/cell lines (Fig. 4B). Using the human retinal RNA, we cloned the LOC387715/ARMS2 cDNA into an expression vector and expressed it in COS-1 (African green monkey kidney fibroblast) cells. Immunoblot analysis reveals a predicted protein band of  $\approx 16$  kDa (12-kDa protein + 4-kDa Xpress epitope) using anti-Xpress and anti-LOC387715/ARMS2 antibodies (Fig. 4C). Subcellular fractionation and costaining patterns of MitoTracker and cytochrome c oxidase subunit IV (COX IV) demonstrate that the expressed LOC387715/ARMS2 protein colocalizes with mitochondrial markers, but not with other organelle markers for endoplasmic reticulum (ER), Golgi apparatus, and lysosomes (Fig. 4D and Fig. 5). Similar results are obtained in the ARPE-19 and JEG-3 cells (data not shown). The treatment of mitochondrial protein fraction, prepared from the transfected COS-1 cells, with Proteinase K results in the loss of LOC387715/ARMS2 and outer membrane proteins (such as translocase of outer mitochondrial membrane 20, Tom20), with no effect on COX-IV, an inner membrane protein (Fig. 4E).

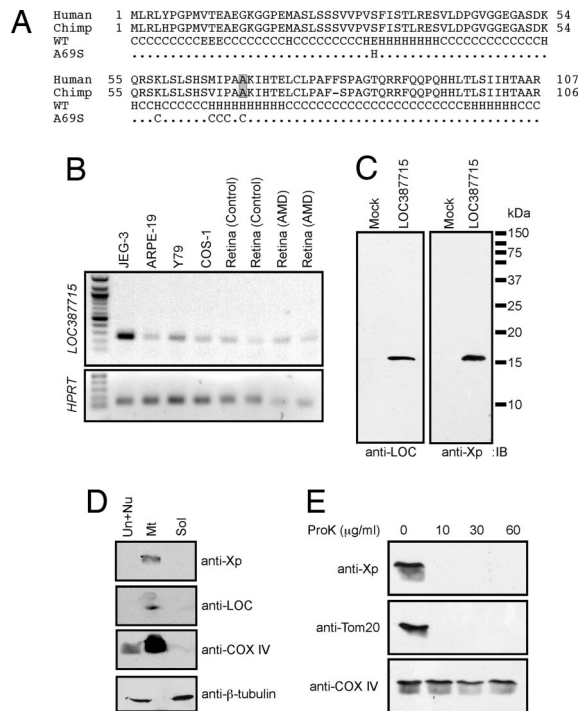
**Discussion**

A strong association between AMD and variants at 10q26 has been confirmed by multiple studies (6, 8, 21, 22, 24, 25). Nevertheless, the identity of the causal variants in the region and the possible

mechanism(s) through which these variants influence disease susceptibility have not been elucidated (8, 21–23). The AMD-associated variants at 10q26 overlap two known genes, PLEKHA1, HTRA1/PRSS11, and a predicted gene LOC387715/ARMS2. Each of these can have a plausible biological relationship to macular degeneration (6). To clarify the genetic association and evaluate possible mechanism(s) of disease susceptibility, we genotyped a panel of 45 tag SNPs that have allowed us to comprehensively assess the impact of variants in the region on AMD. Because SNPs showing the strongest association alter the predicted coding sequence of LOC387715/ARMS2 and are upstream of HTRA1/PRSS11, we carried out further experiments to investigate the biological function of LOC387715/ARMS2 and examine the previously proposed impact of rs11200638 on the expression of HTRA1/PRSS11. Our data enable a direct comparison of HTRA1 and LOC387715/ARMS2 SNPs and provide strong evidence for a single variant of large effect in the region. Specifically, after examining a set of SNPs that tags common variants in the region, we validate the strongest association with rs10490924, a SNP that affects the coding sequence of LOC387715/ARMS2 ( $P < 10^{-29}$ ). Evidence for association is weaker at all other SNPs ( $P > 10^{-21}$ ) and becomes nonsignificant after accounting for rs10490924 in a multiple-regression analysis. The rs11200638 SNP, associated with HTRA1 expression in a previous report and proposed to play a causal role in AMD susceptibility (24, 25), cannot explain the effect of rs10490924 (within the LOC387715/ARMS2-coding region). In contrast, rs10490924 can account for the observed association at rs11200638.

Given that rs10490924 alters the predicted coding sequence of LOC387715/ARMS2, we proceeded to evaluate the gene further. LOC387715/ARMS2 is listed as a hypothetical human gene with a highly conserved ortholog in chimpanzee, but not in sequences from other organisms. The two exons of LOC387715/ARMS2 encode a putative protein of 107 aa, which includes no remarkable motifs, except for nine predicted phosphorylation sites. Consistent with previous reports of LOC387715/ARMS2 expression in the retina and placenta (22), our RT-PCR analysis confirms the presence of LOC387715/ARMS2 transcripts in human retina and a variety of other tissues and cell lines. Furthermore, we can translate LOC387715/ARMS2 cDNA cloned from the human retina, demonstrating that LOC387715/ARMS2 encodes a bona-fide protein.





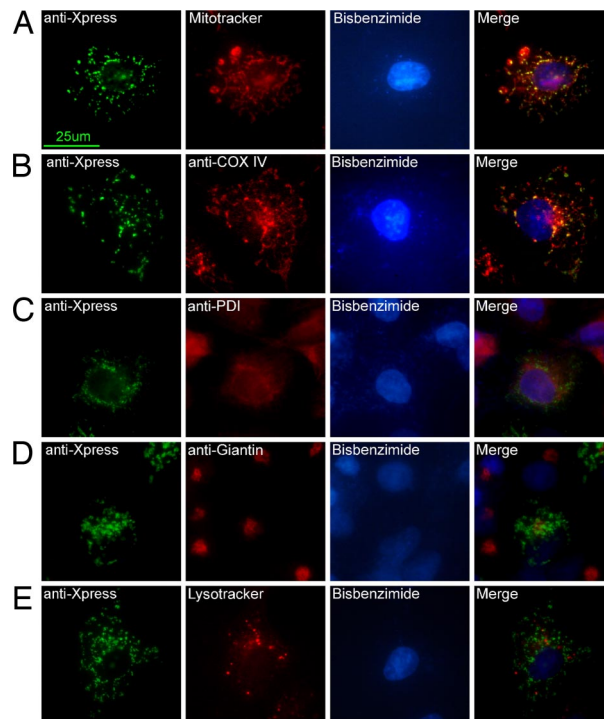
**Fig. 4.** Amino acid sequence and expression of the LOC387715/ARMS2 protein. (A) Amino acid sequence alignment and secondary structure analysis. Line 1, amino acid sequence of the predicted human LOC387715/ARMS2 protein; line 2, chimpanzee LOC387715/ARMS2 sequence; line 3, WT LOC387715/ARMS2 secondary structure prediction (H, helix; E, strand; C, the rest); line 4, secondary structure of LOC387715/ARMS2 altered by the A69S variation (dot, same as WT). The gray box shows Ala codon 69 that is altered by the SNP rs10490924. (B) RT-PCR analysis of LOC387715/ARMS2 transcripts in cultured cell lines and in the retina of control and AMD subjects. *HPRT* was used as a control to evaluate RNA quality and to normalize for the quantity. All PCR products were confirmed by sequencing. (C) Immunoblot analysis of COS-1 whole-cell extracts expressing human LOC387715/ARMS2 protein with N-terminal Xpress-tag. The expressed LOC387715/ARMS2 protein was detected using anti-LOC387715/ARMS2 (anti-LOC) or anti-Xpress (anti-Xp) antibody. (D) Fractionation of COS-1 cell extracts expressing LOC387715/ARMS2. Un+Nu, unbroken cells and nuclear fraction; Mt, mitochondria fraction; Sol, soluble fraction. (E) Proteinase K (ProK) treatment of the mitochondria. The mitochondrial fractions from transfected COS-1 were treated with increasing concentrations of Proteinase K. The antibodies used for immunoblot analysis are indicated.

conclusions. In our real-time RT-PCR analysis, *HTRA1* expression is not significantly different between control and AMD retinas. Together with the genetic association data, these studies show that rs11200638 SNP does not have a major contribution to *HTRA1* gene regulation, and *HTRA1* association to AMD may only be indirect.

In summary, in contrast to the *CFH* gene at 1q32, where multiple SNPs are required to explain the association signal (26), in this report we are able to identify a single SNP (rs10490924) that alone can account for the association of the 200-kb region at chromosome 10q26 with susceptibility to AMD. It is possible that other variants exist in the region, but their identification is likely to require very large sample sizes. Our data further illustrates the challenges of pinpointing causal alleles at susceptibility loci for complex diseases, even in regions where evidence for association is strong and replicated.

## Materials and Methods

**Genotyping and Data Analysis.** We examined 535 affected individuals and 288 unrelated controls that were primarily ascertained and recruited at the Kellogg Eye Center (Ann Arbor, MI) as described



**Fig. 5.** Subcellular localization of the LOC387715/ARMS2 protein. Human LOC387715/ARMS2 cDNA was cloned in pDNA4 vector and transiently expressed in COS-1 cells. The cells were stained with anti-Xpress (green) and an organelle-specific marker (red) as follows. (A and B) MitoTracker (A) and anti-COX IV antibody (B) were used for mitochondria. (C) Anti-PDI antibody was used for endoplasmic reticulum. (D) Anti-Giantin antibody was used for Golgi. (E) LysoTracker was used for lysosome. Bisbenzamide was used to stain the nuclei (blue). (Scale bar, 25  $\mu$ m.)

in refs. 20 and 26. TaqMan assays (ordered from Applied Biosystems, Foster City, CA) were performed at the University of Michigan Sequencing Core Facility. For some SNPs (see *Primers for 10q26 SNPs That Were PCR-Amplified and Sequenced in SI Text*), PCR was used for amplification before sequencing. In a follow-up experiment, we genotyped a set of 20 overlapping markers (including rs10490924) by using an Illumina (San Diego, CA) Golden Gate panel; a comparison with our original calls revealed an overall error rate of  $\approx 1.0\%$ , which did not differ between cases and controls. The Illumina genotypes (with an overall completeness of 98.9%) support our conclusions, showing much stronger association for rs10490924 than for any other marker in the region and showing that rs10490924 can explain observed results for all other SNPs. However, we report the TaqMan data, despite the lower completeness, because it includes a larger number of SNPs in the region.

Genotypes were checked for quality by examining call rates per marker and per individual and by calculating an exact Hardy-Weinberg test statistic (39). After excluding individuals with  $< 25$  successfully typed SNPs, a total of 280 controls and 466 cases were selected for analysis. The average genotyping completeness was 94.3%. Genotype frequencies between cases and controls were compared using standard  $\chi^2$  tests and a model-based procedure (26, 28). To evaluate multiple SNP models, we first imputed missing genotypes as recommended (40).

**RT-PCR Analysis.** Human retina tissues were procured from National Disease Research Interchange (Philadelphia, PA). Total RNA from retinas of four adults, each with AMD (ages 60–93 yr) or without any maculopathy (ages 64–100 yr) was reverse transcribed per standard protocols (41). Quantitative PCRs were performed in triplicate with Platinum Taq polymerase (Invitrogen, Carlsbad,



CA) by using the iCycler iQ Real-Time PCR Detection System (Bio-Rad, Hercules, CA). SYBR green I (Invitrogen) was used for detection, and results were analyzed by the  $\Delta\Delta C_t$  method using *HPRT* for normalization. All primers are listed in *Primer and Oligonucleotide Probe Sequences* in *SI Text*.

**Plasmid Construction and Mutagenesis.** Three regions of the *HTRA1* promoter (−3,652 to + 57, −775 to + 57, and −425 to + 57) (GenBank accession no. AF157623) were subcloned into pGL3-basic vector (Promega, Madison, WI). The full-length *LOC387715/ARMS2* (XM.001131263) cDNA was amplified from human retinal RNA by RT-PCR and cloned into pcDNA4 His/Max C vector (Invitrogen). The QuikChange XL site-directed mutagenesis kit (Stratagene, La Jolla, CA) was used to generate all mutants of the *HTRA1* promoter and *LOC387715/ARMS2* expression construct.

**EMSA.** Nuclear extracts from bovine retina were used for EMSA per standard protocols (41). In supershift experiments, antibodies against AP-2 $\alpha$  and SP-1 (Santa Cruz Biotechnology, Santa Cruz, CA), and NRL (a retina-pineal specific transcription factor) (42) were added after the incubation of <sup>32</sup>P-labeled oligonucleotides with retinal nuclear extract.

**Antibody Generation.** Rabbit anti-LOC387715/ARMS2 polyclonal antibody was raised against the linear peptide sequences <sup>47</sup>GGE-GASDKQRSKL<sup>59</sup> and <sup>87</sup>QRRFQQPQHHLTSL<sup>100</sup>, derived from the predicted human LOC387715/ARMS2 protein (XP.001131263).

**Transfections, Protein Analysis, and Immunocytochemistry.** Cells were cultured according to standard procedures and transfected at

80% confluency with plasmid DNA by using FuGENE6 (Roche Applied Science, Indianapolis, IN). For luciferase assays, each plasmid containing pGL3-*HTRA1* WT or SNP (0.5  $\mu$ g per well) was cotransfected with cytomegalovirus- $\beta$ -galactosidase (0.1  $\mu$ g per well) plasmid to normalize for the amount of DNA and transfection efficiency, and the reporter activity was measured by a kit from Promega. Transfections were done in triplicate and repeated three times. Cell extracts were subjected to immunoblotting using mouse monoclonal anti-Xpress antibody (Invitrogen), rabbit anti-cytochrome *c* oxidase IV (COX IV) (Abcam, Cambridge, MA), or rabbit anti-Tom 20 antibody (Santa Cruz Biotechnology), according to the standard protocols (43).

Fractionation of COS-1 cell extracts was performed as described in ref. 44. In some experiments, the mitochondrial fraction was treated with Proteinase K for 3 min at 26°C. Immunostaining was performed as described in ref. 45 by using anti-Xpress antibody, MitoTracker and LysoTracker (Molecular Probes, Eugene, OR), rabbit anti-cytochrome *c* oxidase IV (COX IV) and rabbit anti-Giantin (Abcam), and rabbit anti-protein disulfide isomerase antibody (PDI) (StressGen Biotechnologies, Victoria, Canada).

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